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ALLELIC RELATIONSHIPS AND REVERSE MUTATION IN ESCHERICHIA COLI

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MANY aspects of mutation in bacteria have been studied by mutational patterns, biochemical diversity, and other phenotypic determinations without recourse to breeding test (LURIA 1947). The recombination techniques that have been developed in *Escherichia coli* K-12 (TATUM and LEDERBERG 1947) permit a more incisive analysis of the bacterial genotype. A series of lactose-negative (Lac^-) mutants in strain K-12 which revert at different rates has provided experimental material for the study of reverse mutation, the genetic factors controlling the different mutation rates, and allelic multiplicity at the Lac_1 locus.

MATERIALS AND METHODS

The intercrossable cultures used in these experiments were ultimately derived from the two auxotrophic lines of K-12: 58-161 (B^-M^-, biotinless, methionineless) and Y-10 ($T^-L^-Th^-$, threonineless, leucineless, thiamineless) in which each nutritional factor had been obtained in a discrete mutational step (TATUM 1945; TATUM and LEDERBERG 1947). Liquid cultures were grown in Difco penassay broth, while stocks were maintained on nutrient agar slants. The plating media used consisted primarily of EMB (a complete eosin-methylene blue agar) and EMS (a minimal modification of EMB), containing one percent sugar. The media were prepared from dry mixes to which water, the appropriate sugar, and other supplements were added before sterilization in the autoclave. On EMB and EMS agar fermenting colonies are opaque purple while nonfermenters are lightly tinted. Thiamine was often added to the minimal crossing media to relax selection for Th^+ (see LEDERBERG 1947). Details of media and various methods are given elsewhere (LEDERBERG 1950a; LEDERBERG *et al.* 1951). Crosses were usually carried out on EMS lactose agar for the direct scoring of fermentative characters by inspection of the colonies. After 48 hours incubation, the prototroph progeny were picked and streaked on EMB lactose to provide purified colonies for further tests.

A 125 watt Hanovia high pressure mercury vapor arc emitting a variety of wave lengths was used for ultraviolet exposures because of its high intensity output. Most of the irradiations were made directly on EMB lactose agar plates prespread with a drop of a 20 hour broth culture containing about

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10^9 cells per ml. Each plate was exposed for 7–10 seconds at a distance of 15 cm, resulting in approximately six decades of killing. The irradiations were conducted in this fashion for the empirical purpose of securing a variety of fermentation mutants.

In earlier experiments many lactose-negative mutants were intercrossed. It soon became apparent that a number of distinct loci were implicated among the different mutants which shared the lactose-negative phenotype (LEDERBERG 1948a). The most common locus was designated Lac_1^- and was selected for the present study. The principal stocks are summarized in table 1. The

TABLE 1

List of stocks.

$T^-L^-Th^-$	Genotype	Phenotype on lactose	Source	Agent
Y-10	Lac^+	wild type	679-680	X-ray
Y-53	Lac_1^-	semi-mutable	Y-10	U-V on lactose agar
Y-70	Lac_1^-	stable	Y-53	U-V on lactose agar
W-112	Lac_1^-	stable	Y-10	U-V on lactose agar
W-133	Lac_7^-	mutable	Y-10	U-V on lactose agar
W-716		Lac^+ unstable	Y-70	spontaneous
W-844	Lac^-	mutable	W-716	spontaneous
W-888	Gal_1^-	Lac slow	W-750 × W-588	segregation from Lac^-Gal^-/Lac^+Gal^+ diploid
W-902	Gal_2^-	Lac^+	Y-10	U-V on galactose
W-1282	Lac_1^-	semi-stable	Y-53	spontaneous
B^-M^-				
Y-40	Lac^+V1^r	wild type	58-161	selection on T1
Y-87	Lac_1^-	mutable	Y-40	N-mustard
W-516	$Lac_1^-Pur^-$	stable	Y-87	U-V on lactose agar
W-744	$Lac_1^-Mal^{sl}$	stable	Y-87	U-V on lactose agar
W-750	$Lac_1^-Gal_1^-$	stable	Y-87	U-V on lactose agar
W-811	$Lac_1^-Gal_4^-$	stable	Y-87	U-V on lactose agar
W-842	Lac_1^-	stable	Y-87	spontaneous
W-1306	Lac^+V6^r	wild type	Y-40	selection on T6
W-1307	$Lac_1^-V6^r$	mutable	Y-87	selection on T6
W-1435	$Lac_1^-Mal^-Mtl^-V1^rV6^r$	semi-mutable	....	segregation from H-1; then selection on T6 (see Lederberg, 1949)
$B^+M^+T^+L^+$				
W-946	$Lac_1^-Gal_2^-$	semi-mutable	W-902 × Y-87	recombination

Lac_1^- designation means that preliminary tests revealed no lactose-positive recombinants in crosses of pairs of Lac_1^- mutants.

In addition to color differences of colonies on EMB or EMS lactose agar, Lac^+ and Lac^- can be distinguished in Durham tubes containing a lactose-peptone broth with bromcresol purple indicator. The change from a purple to a yellow color and the evolution of gas as end products in fermentation are observed for Lac^+ inocula. Lac^- cells give a corresponding turbid growth but the original alkaline color persists.

A test of greater precision affords a measurement of the adaptive "lactase," β-D-galactosidase (LEDERBERG 1950b). Nitrophenol is measured photometri-

cally after a short period of incubation of the chromogenic substrate, *o*-nitrophenyl β-D-galactoside with washed cells. The capacity to produce an adaptive lactase after growth in lactose broth has been lost in *Lac*$^-$ mutants. However, a small residual activity is characteristic of *Lac*$_1$$^-$ cultures. The lactose analog, *n*-butyl-β-D-galactoside, can be fermented by this group of mutants and can evoke the adaptive formation of galactosidase in amounts comparable to that formed by *Lac*$^+$. It may be noted that this substrate appears to select for

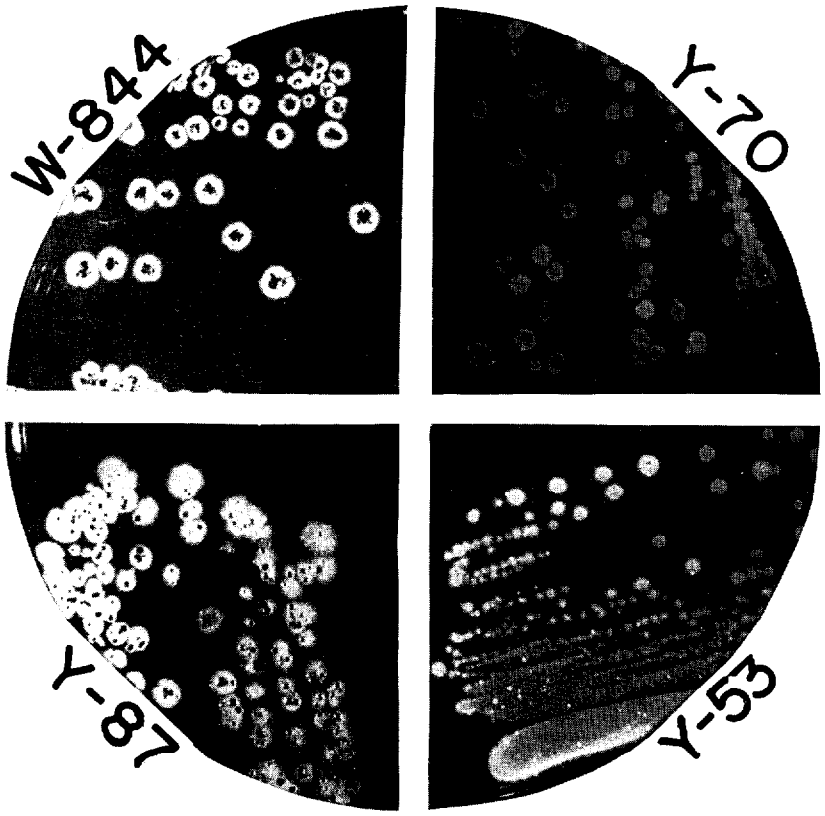

FIGURE 1.—Papillating colonies from EMB lactose agar streak plates.

spontaneous lactase-negative mutants, presumably by virtue of the toxicity of enzymatically released butanol.

PAPILLAE AND REVERSE MUTATION

Several *Lac*$^-$ strains develop outgrowths or papillae within the colonies, or marginal sectors, of the typical *Lac*$^+$ color on EMB lactose agar (figure 1). Stable *Lac*$^+$ cultures indistinguishable from type are obtained from these papillae. Such papillate forms ("*Bacterium coli mutabile*") provided one of the earliest recognized and most cited examples of mutation in bacteria (MASSINI 1907; HADDOW 1937; MONOD and AUDUREAU 1946; LURIA 1947).

The Lac^+ papillae may represent reverse mutation to the wild type allele or mutation at another "suppressor" or "mimic" locus. To discriminate between these alternatives it is necessary to isolate the reversions and cross them with a wild type Lac^+ strain. If the Lac^+ derived from papillae are mimics of wild type, the progeny will include Lac^- recombinants, their number depending upon the closeness of the linkage between the Lac_1 and the mimic locus.

Accordingly, a single papilla was fished from each of 21 colonies of the mutable strain, Y-87 ($Lac_1{}^{-m}$). Each of the secondary colonies was carefully purified by successive streakings to rid them of adherent Lac^- cells, and five more successive single colony transfers were made after homogeneity was established. Each reversion was crossed several times with the appropriate Lac^+ (Y-10) on EMS lactose thiamine agar until about 1,000 recombinants

TABLE 2

Papillation frequencies for $Lac_1{}^-$ mutants.

Strain	No. of colonies	Mean No. of papillae	Chi-square* and DF	Probability
W-1282	100	0.61**	0.05_1	.8
Y-53	151	1.1	3.39_2	.18
	95	2.4	3.33_4	.51
	100	2.4**	10.4_4	.04
	97	3.0	3.18_5	.68
	289	4.0	18.47_5	.002
	125	4.4	3.22_6	.90
Y-87	109	4.0	11.45_6	.76
	204	6.1	5.49_8	.70
	100	14.0**	....	....

*For goodness of fit to a theoretical Poisson distribution of the same mean with n–2 degrees of freedom.

**Same experiment.

had been examined. Eventually, a total of 31,523 recombinants had been observed. One stable Lac^- exception was found in the cross Y-87R5 × Y-10. The cross was repeated but no other Lac^- appeared, and the exception might well have been a spontaneous mutation. In view of the infrequency of Lac^- among these prototrophs, the change of Lac^- to Lac^+ in Y-87 most probably constitutes a reverse mutation at the Lac_1 locus. Similar isolations of papillae were made from single colonies of Y-53 but the genetic tests were not as exhaustive. None of the 4,784 recombinants of the derived Lac^+ with Y-40 was distinguishable from wild type.

In contrast to the mutable Lac^- strains, no reverse mutants have been demonstrated for the more stable strains, even after prolonged selection. Twenty derivatives isolated from old cultures of W-112 ($Lac_1{}^{-st}$) on lactose agar or broth were all slow lactose fermenters (Lac^{sl}). Three classes of prototrophs were recovered in crosses of Lac^{sl} with Lac^+ (Y-40): Lac^+, Lac^{st}, and Lac^{-st}. The Lac^{sl} types are therefore not reversions of Lac_1 but partial

mimics or suppressors. During this study several other suppressors of Lac_1^- mutants encompassing a wide range of fermentative ability were discovered.

The absence of mimic mutations among the mutable strains Y-87 and Y-53 is only apparent. The frequent and early production of reverse mutants in Y-87 and Y-53 would exclude the development and detection of the less frequent suppressors, especially when the reversions ferment lactose more vigorously. Where reverse mutation does not occur, the selection of Lac^{sl} mimics is facilitated.

On two separate occasions a colony of Y-70 and W-112 yielded a single normal Lac^+ papilla at the 48 hour stage. One of these carried a coincidental mutation for parathiotrophy. This culture, W-716, proved to be an extremely unstable Lac^+. When maintained on nutrient agar stock slants, it gave rise to a variety of Lac^- and Lac^{sl} types. Lactose agar delayed but did not prevent the accumulation of Lac^- cells. The Lac^- colonies themselves were unstable, including a wider divergence of distinct patterns than had previously been encountered. Of 1300 colonies examined in one derived culture, W-719, five contained a single papilla representing probably the lowest perceptible mutation rate among these cultures. The colonies of a second derivative, W-844 (figure 1), contained an average of well over 25 papillae so that counts were unreliable. The Lac^+ papillae isolated from any of these forms resembled W-716 both in their instability and the constellation of Lac^- subtypes.

The genetic position of W-716 has not yet been satisfactorily settled due to the extreme difficulty in separating mutant from recombinant Lac^-. The standard test for reverse mutation was carried out with the *V6* marker (resistance to phage T6, closely linked to Lac_1, LEDERBERG 1947) in the wild type tester (W-1306) to aid in the identification of recombinant prototrophs. About 2 percent of the latter were Lac^-. The stable Lac^+ recombinants were $V6^r$; the unstable $V6^s$, thus showing a close correlation with the parental coupling. Five single colonies of both these types were transferred to nutrient agar slants and sampled about a year and a half later. Lac^-/Lac^+ mixtures were found only in the populations classified as $V6^s$. This linkage locates the unstable mutant W-716 at or near the Lac_1 locus whose expression is modified.

MUTABILITY PATTERNS

The rate of formation of lactose-positive papillae has so far been the most characteristic index of subdivision among these otherwise phenotypically indistinguishable cultures. Representative examples of four established categories are, in order of revertibility: Y-70 and W-112, W-1282, Y-53, and Y-87 (figure 1). The frequency distribution of Lac^+ papillae was studied in this group of strains. Platings from appropriate dilutions of either 24-hour colonies on agar or broth cultures were made so as to give no more than 15 evenly distributed Lac^- colonies on each plate. Lac^+ papillae began to appear after 36–48 hours of incubation and continued to emerge as the colonies aged. The classification of colonies with 0, 1, 2, ... n papillae was recorded at the 48

TABLE 3

Test for recombination of mutability: Lac$^+$ × Lac$^-$

Cross	Lac$^+$ × Lac^{-m}	Lac$^+$ × Lac^{-st}	
	Y-10 × Y-87	Y-40 × W-112	Y-40 × Y-70
Total number of prototrophs	3,188	1,097	3,905
Percent Lac$^-$	23.2	61.3	71.3
Number Lac$^-$ tested for papillation	551	305	105
Percent Lac$^-$ stable	0	100	100

hour stage. Under these conditions, the observed numbers of papillae per colony often showed a good fit to a theoretical Poisson distribution with the same mean (table 3, figure 2). The data are consistent with the hypothesis of equally probable and independent mutations in every mutable colony.

Deviations from the theoretical distribution were also observed, especially after 48 hours. An individual strain also showed fluctuation about the mean

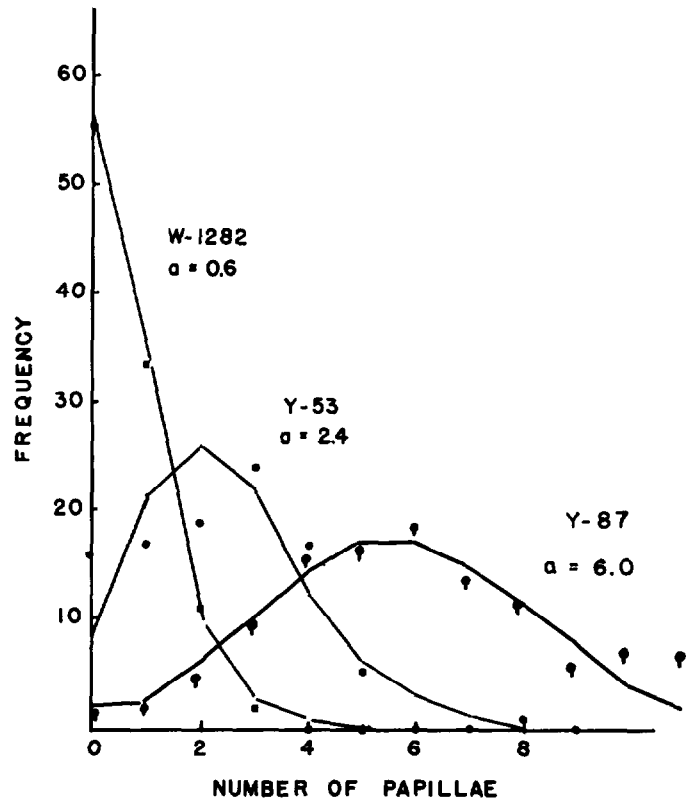

FIGURE 2.—Theoretical frequency distribution of papillae expressed as numbers of colonies with 0, 1, 2, . . . n Lac$^+$mutants for the observed mean in mutable Lac$^-$ strains. The points represent the observed values.

from experiment to experiment with occasional wide discrepancies from the Poisson distribution. These disparities probably reflect uncontrollable variations in local environment, and correlations between changes in growth rates of the Lac^- mother cells, the mutation rate, and the capacity of the mutants to be manifested as papillae. Nevertheless, each strain retained its relative position in the mutability series.

Intercrosses were made between mutable × stable and mutable × semistable to ascertain whether a clear-cut genetic difference was involved. Among the 220 Lac^+ prototrophs from $B^-M^-Lac^-$ mutable (Y-87) × $T^-L^-Lac^-$ stable (W-112), there were only two sharply defined classes of segregants, resembling the papillation patterns of the parents. Sixty-six percent were stable, which is in fair agreement with the proportions expected from the established linkages of the nutritional markers and Lac_1. Crosses of the intermediate

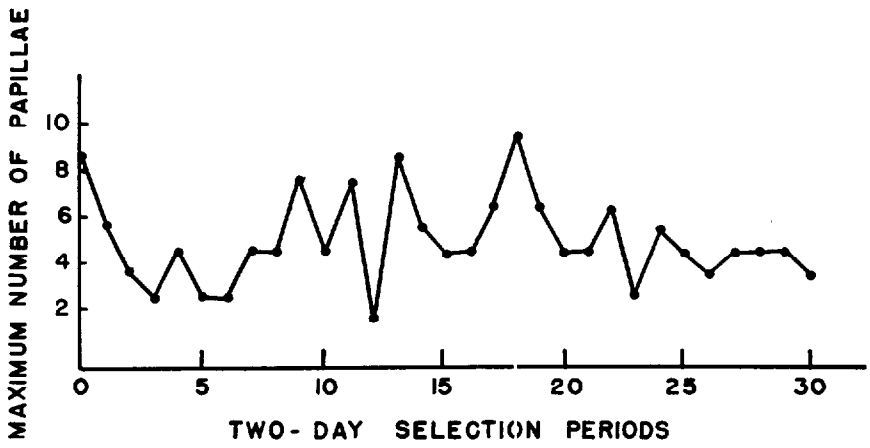

FIGURE 3.—Fluctuations in the maximum number of papillae per colony of Y-53.

alleles, Y-53 or W-1282 × Y-87 types, yield results more difficult to interpret. Individual nonpapillate prototroph colonies might represent either a genetically stable recombinant or the extreme class of a random distribution. Within this limitation, the recombinants all concorded with one or the other parent.

Spontaneous changes in mutability patterns have all led to a reduction rather than enhancement of papillation. Stable, nonpapillate colonies have been derived from Y-53 and Y-87. W-1282 of spontaneous origin occupies a mutability level intermediate between the parental Y-53 and the completely stable types. In an attempt to augment the mutability of Y-53 by recurrent selection, serial platings were made using the colony with the most papillae among 50–200 colonies at each two day selection interval. This selection was extended over thirty such stages, but no consistent trend in mutability was observed. Figure 3 illustrates the stability of the papillation pattern of this strain.

Crosses of the various Lac^- with Lac^+ were carried out to establish whether the strains constitute a series of multiple alleles at the Lac_1 locus or

whether a mutability modifier is involved. The latter hypothesis predicts the occurrence of both stable and mutable recombinants among the Lac^- progeny. Because only parental types have been recovered in such crosses, the argument that the differences in mutability reside within the locus is favored (table 3).

Before the conclusion can be drawn that the alleles differ in their intrinsic revertibility one further possibility must be considered. Lac^+ reversions may occur at a constant rate but the differences in papillation reflect differences in the selection and expression of Lac^+ mutants. This interpretation may be tested by determining whether large populations of Lac^{-st} mutants are able to suppress the appearance of small numbers of Lac^+ cells when inoculated together in lactose medium. In nutrient broth where there is no obvious basis for selective differences, the growth rates of Lac^{-st} and Lac^+ are indistinguishable. Six lactose indicator broth tubes were inoculated with a mixture of approximately 2 Lac^- and 3×10^8 Lac^{-st} (W-842) cells. Each tube turned yellow after 48 hours as a result of the development of a lactose-fermenting population. Lac^- cells therefore compete effectively against preponderant populations of Lac^{-st} in media favoring the growth of Lac^+. Similar conclusions were reached by KRISTENSEN (1944) in a more elaborate study.

INDUCED ALTERATIONS OF MUTABILITY

The mutability of a gene may be controlled by other loci (RHOADES 1941). To discover genic regulators of mutation rate, large populations of Lac_1^- cells were subjected to ultraviolet irradiation. The surviving colonies were inspected for deviations in papillation pattern. Mutable strains were examined for non-papillating whole colonies or sectors, and similarly, Lac^+ sectors or papillae were looked for among stable strains. The suspected mutants were isolated, purified, and checked for the genetic markers of the parent strain.

Systematic study of over 33,000 survivors from a series of irradiations of the stable strains (Y-70, W-112, and W-842) yielded no examples of either mutable colonies or reverse mutants. On the other hand, stable substrains were obtained from treated populations of the mutable Y-53 and Y-87. The induced stable strains of Y-87 were studied in greater detail. Outcrosses with Lac^+ (Y-10) separated the new strains into two groups: one involving allelic or pseudoallelic changes in mutability (I) and the other consisting of forms in which a second locus was involved (II). The basis of the classification depended upon whether only parental types of prototrophs were recovered (I) or whether Lac^- mutable colonies also appeared among the progeny (II).

The majority of the mutants belonged to the first category. They were further characterized by the retention of fermentative activity on butyl galactoside and by stability on lactose throughout the manipulations to be discussed below. Each member of group II ($Lac_1^-X^-$) failed to ferment butyl galactoside. The capacity for reverse mutation could be restored by replacing the modifier with X^+ by recombination or mutation.

In most of the derived stable forms of this group, the "*X*"-modifier exhibited its own phenotypic effects that facilitated its characterization. Alternatively, the modifier could be extracted in combination with *Lac*$^+$. The resynthesis of *Lac*$^{-m}X^-$, detected as stable recombinants in progeny tests of possible *Lac*$^+X^- \times Lac^{-m}X^+$ identify the extracted modifier. In order to accomplish the repeated crossings, advantage was taken of auxotrophic segregants from persistent diploids (LEDERBERG 1949).

One type of mutability modifier was associated with a nutritional requirement for purine plus thiamine in W-516 (*Pur*$^-$). The components of this combination were not separable by reverse mutation. Papillation was restored in *Pur*$^+$ reversions. The growth of *Pur*$^-$ colonies was restricted on EMB medium which is deficient in nucleic acid components. Moreover, glucose itself is less rapidly fermented by W-516 than Y-87 grown on such media. A study of the relationship of *Lac*$_1^{-m}$ and *Pur*$^-$ disclosed that the effects on mutability were indirect. The *Lac*$^+$*Pur*$^-$ recombinants obtained in crosses of Y-10 $\times$ W-516 were fermentatively weak. The inability of a *Lac*$^+$*Pur*$^-$ genotype to express itself normally is a clue to the absence of *Lac*$^+$ papillae in W-516 colonies. It is reasonable to conclude that the *Pur*$^-$ gene depresses glycolysis, consequently removing the selective advantage of *Lac*$^+$ mutants as confirmed in reconstructed mixtures of *Lac*$^+$*Pur*$^-$ and *Lac*$^-$*Pur*$^-$. There is no evidence that the mutation rate itself has been altered by the introduction of *Pur*$^-$ into Y-87. The *Pur*$^-$ mutation has been noticed in many subsequent irradiations of *Lac*$^+$ and *Lac*$^-$ as a small, thin colony. A similar situation was found in W-744, where the second mutation showed reduced fermentation of maltose, mannitol, and galactose.

The coincidence of a *Gal*$^-$ mutation in a *Lac*$^-$ stable strain derived from Y-87 was first recognized in W-750. Verified *Gal*$^+$ reversions were recovered as papillae on EMB galactose agar after several days of incubation. These *Lac*$^-$*Gal*$^+$ derivatives again produced papillate colonies on lactose, showing that the replacement of *Gal*$^+$ for *Gal*$^-$ restored the mutability of *Lac*$^-$ to *Lac*$^+$. The *Lac*$^+$ papillae were isolated and confirmed as back-mutants of *Lac*$_1^-$. Other galactose fermenters were found which were stable on lactose. Backcrosses to wild type proved these exceptions to be mutations at mimic loci rather than reverse mutants of *Gal*$^-$. Alleles at this locus which did not interact with *Lac*$_1^-$ were not found.

All four combinations of *Lac*$^{+/-}$ and *Gal*$^{-/-}$ were recovered from the cross W-750 $\times$ Y-10. Among 41 *Lac*$^-$ all the *Gal*$^-$ were stable, while *Gal*$^+$ papillated on lactose. Preliminary linkage tests place this *Gal* locus between (*BM*) and *Lac*$_1$. The *Gal*$^-$ mutation was extracted from the double mutant by crossing W-750 with a suitable *Het* stock (LEDERBERG 1949). Auxotrophic *Lac*$^+$*Gal*$^-$ segregants for use in crosses were obtained via a persistent diploid. In the cross Y-87 $\times$ W-888 (*Lac*$^-$*Gal*$^+ \times$ *Lac*$^+$*Gal*$^-$), which may be contrasted with W-750 $\times$ Y-10 (*Lac*$^-$*Gal*$^- \times$ *Lac*$^+$*Gal*$^+$), stable *Lac*$^-$ reappeared as *Gal*$^-$*Lac*$^-$ recombinants. Reversion to *Gal*$^+$ restored the revertibility of

these prototrophs on lactose agar. The same types appeared in crosses of Y-53 × *Lac*$^+$*Gal*$^-$.

The *Gal*$^-$ might influence the mutation rate of genetically susceptible alleles at the Lac_1 locus or it might affect the ability of *Lac*$^+$ mutants to express themselves as visible clones in the mother colony. The recombination analysis cannot decide between these alternatives. The second interpretation, however, is compatible with physiological tests of the genotypes. The *Gal*$^-$ allele slightly modified the appearance of *Lac*$^-$ toward *Lac*$^+$ and the *Lac*$^+$ somewhat toward slow fermentation. Galactosidase assays were made on cells of various genotypes grown in a lactose-peptone broth. The values per unit dry weight, expressed in relation to wild type K-12 as 100 percent are: Y-87 ($Lac_1^-Gal^+$) 3 percent; W-750 ($Lac_1^-Gal_1^-$) 20 percent; W-946 ($Lac_1^-Gal_2^-$) 18 percent; W-811 ($Lac_1^-Gal_4^-$) 25 percent; W-888 ($Lac^+Gal_1^-$) variable, 50–100 percent; W-902 ($Lac^+Gal_2^-$) 90 percent. The galactosidase assays of the *Lac*$^-$ combinations correspond reasonably well with their appearance on EMB lactose agar. The *Lac*$^+$*Gal*$^-$ types, however, appear much weaker than these assays would indicate. A loss of efficiency of lactose fermentation due to the nonfermentability of the galactose residue may explain why less acid is produced from lactose in lactose broth indicator tubes, despite the retention of lactase activity. The accumulation of monosaccharide during the fermentation of lactose by *Lac*$^+$*Gal*$^-$ but not *Lac*$^+$*Gal*$^+$ cells has been demonstrated by Barfoed's monose assay method (J. LEDERBERG, unpublished).

These results suggest that the competitive advantage on lactose of *Lac*$^+$ over *Lac*$^-$ is modified in *Gal*$^-$ genotypes. This possibility was explored by reconstitution of the selective conditions under which *Lac*$^+$ papillae were observed. *Lac*$^+$*Gal*$^+$ introduced into lactose broth, although outnumbered 10 to 100 million-fold by *Lac*$^-$*Gal*$^+$ cells, were detected after 24–48 hours, showing intense selection for *Lac*$^+$. Comparable inoculations with *Gal*$^-$ strains failed to demonstrate any preponderance of *Lac*$^+$ within that period unless they had been present initially in a ratio of 1 *Lac*$^+$: 1000 *Lac*$^-$.

The specificity of *Gal*$^-$ genes on apparent mutability of *Lac*$^-$ was studied with several nonallelic *Gal*$^-$ mutants. One of these was obtained originally as a semistable colony from ultraviolet irradiation of Y-87. This $Gal_2^-Lac^-$ had a papillation pattern resembling Y-53. Gal_4^- and Gal_5^- gave stable *Lac*$^-$ recombinants similar to Gal_1^- when crossed with Y-87. Gal_1^- also suppressed the apparent mutability of a papillate stock carrying a mutation at another *Lac* locus (Lac_7^-).

Combinations of $Lac_1^-Gal_1^-$ or $Lac_1^-Gal_2^-$ strains show a characteristic interaction with Lac_1^- on EMB lactose agar. A stronger fermentation reaction occurred at the junction of cross-streaked inocula after 1–2 days incubation. Subculture from the lactose-positive area gave only the original *Lac*$^-$ types, and a new synergistic reaction at the juxtaposition of similar colonies. The Lac_1^- mutant has already been diagnosed as producing lactase in response to butyl galactoside but not to lactose itself (LEDERBERG 1951). The influence

of the *Gal*$^-$ mutation and the synergism of *Gal*$^-$ with *Lac*$_1$$^-$*Gal*$^+$ mutants reinforce the conclusion that the *Lac*$_1$$^-$ mutants retain a considerable lactase-forming competence not ordinarily realized in the presence of lactose.

The disposition of modifiers that lack their own phenotypic effects is more difficult. The fact that all of the stable derivatives in this class lost the ability to respond to butyl galactoside suggests the possibility that a second *Lac* mutation, at another locus, had been introduced. Even though both mutations of a double *Lac*$^-$ mutant, *Lac*$_1$$^-$*Lac*$_x$$^-$, were potentially revertible, their combination would be stable as far as detectible reversions to *Lac*$^+$ is concerned. Reversion at one locus would be concealed by the mutant status of the second.

This suspicion was verified by the production of a *Lac*$_1$$^-$*Lac*$_7$$^-$ double mutant by recombination of two papillate single mutants. The double mutant was nonpapillate. Its composite nature was confirmed by crosses to wild type which separated and re-exposed the revertible components.

The status of the stabilized Y-87 derivatives that fall into this category remains in doubt, pending systematic tests for the identity of the presumed *Lac*$_x$$^-$ by crosses to standard *Lac*$^-$ mutants at various loci. Unfortunately, not all of the stocks needed for this enterprise have yet been developed in suitable form. Nevertheless, the modification of response to butyl galactoside as well as papillation of these derivatives argues against the participation of a true mutability modifier similar to *Dotted* in maize.

PSEUDOALLELISM AT THE *Lac*$_1$ LOCUS

Evidence for separating a group of four types of *Lac*$_1$$^-$ strains on the basis of their intrinsic mutation rate to a fifth allele, *Lac*$^+$ has been offered. The observed effects appeared to reside in that locus alone. However, the identification of mutant factors as alleles is at best a negative inference, derived from the failure to detect nonparental classes in a large series of tests. The scope of such experiments is subject to technical limitations inherent in the material handled (cf. STADLER 1951) and the spontaneous mutation rate of the crucial locus. Because of the applicability of selective techniques for screening large numbers, bacteria have special advantages for such studies.

In an extension of the preliminary allelism tests already cited, the crosses of *Lac*$^-$ mutants were repeated on a large scale. The presumed *Lac*$_1$ locus has been dissected into at least two components. Whereas no *Lac*$^+$ recombinants were observed among 60,000 prototrophs from Y-53 × Y-87, about 1 in 1500 from Y-87 × W-112 was lactose-positive. To avoid confusion with spontaneous reversions in the *Lac*$^-$ mutable parent, certain precautions were taken: the *Lac*$^-$ parents were freshly reisolated from a nonlactose medium to minimize the accumulation of *Lac*$^+$ mutants.

Because of its close linkage to *Lac*$_1$ (LEDERBERG 1947), *V6* served as a useful marker. Of 14 *Lac*$^+$ prototrophs from a total of 25,000 observed in a cross of W-1307 (*B*$^-$*M*$^-$*I'6*^{r}*Lac*$^{-m}$ × *T*$^-$*L*$^-$*I'6*^{s}*Lac*$^{-st}$) 11 were *V6*^{s} like the *Lac*$^-$ stable parent. In a parallel control experiment, of a reverted W-1307 × W-112,

all of the 24 Lac^+ prototrophs tested were $V6^r$. It may be concluded that the exceptional lactose-positive prototrophs originated by recombination rather than spontaneous mutation. Y-87 and Y-53 may therefore be considered as Lac_{1a}^- and W-112 as Lac_{1b}^-. The sequence $BM \ldots V6 \ldots a \ldots b \ldots TL$ is indicated by these data. For further critical study, a second marker just to the right of $Lac_{1a,b}$ would be very useful, but has not yet been found.

Studies on heterozygous compounds of Lac_{1a} and Lac_{1b} suggest a very close relationship between these components. W-112 ($V6^s a^+ b^- V1^s Mtl^+$) was crossed on EMS mannitol agar with a filial Y-53 derivative, W-1435 ($V6^r a^- b^+ V1^r Mtl^- Het$). *Het* is a factor that promotes nondisjunction (LEDERBERG 1949). Heterozygous diploid prototrophs were isolated on the basis of their segregation for *Mtl*, *V1* and *V6*. Such diploids are presumably a^+b^-/a^-b^+ at the Lac_1 locus. Although they showed little or no fermentation of lactose, this constitution was verified by the segregation of Lac^- stable ($V6^s a^+ b^-$) and mutable ($V6^r a^- b^+$) haploids. Crossing over to yield a^+b^+ occurs relatively frequently and results in marked papillation. Heterozygotes which are almost certainly a^+b^+/a^-b^- occur among exceptional lactose-positive prototrophs from the same cross. In distinction to the opposed "repulsion" heterozygotes, these "coupling" diploids segregate lactose-positive and stable lactose-negative haploids. The latter are assumed to be a^-b^-, complementary to the a^+b^+ crossovers. Because the presumed a^+b^+/a^-b^- is lactose-positive while a^+b^-/a^-b^+ is lactose-negative, a "position effect" is suggested.

A group of mutants has been designated as Lac_4^-. These mutants differ from Lac_1^- in showing little or no residual lactase when grown on butyl galactoside, and when crossed with Lac_1^- give lactose-positive recombinants with a frequency of 0.1 to 0.2 percent (LEDERBERG 1951). Crosses of Lac_4^- with W-112 (a^+b^-) or Y-53 (a^-b^+) have given identical results. In addition to the rare Lac^+ crossovers, lactose-positive nondisjunctional exceptions were found (LEDERBERG 1949). In contrast to compounds of a^-b^+/a^+b^-, heterozygotes of the constitution $Lac_4^- a^+b^+/Lac_4^+ a^-b^+$ and $Lac_4^- a^+b^+/Lac_4^+ a^+b^-$ approach Lac^+ in their lactase activity. In spite of the close linkage of Lac_1 with Lac_4, these loci appear to function independently of each other and are therefore assigned different symbols.

DISCUSSION

Multiple alleles can be characterized by differences in mutability as well as in physiological expression. For example, two inositol-dependent mutants have been described in Neurospora that represent allelic forms indistinguishable from one another except for their capacity to revert to wild type upon suitable mutagenic treatment (GILES 1948). The mutability difference segregated in the ascospore progeny when the two mutants were crossed.

Recurrent mutants with different rates of spontaneous reversion were identified at the Lac_1 locus in *E. coli*. Qualitative examination disclosed four

categories. Additional intergrading alleles were suspected but may be unclassifiable by ordinary means. The number of possible configurations at this locus may be very large, although empirical tests for precise separation of all the potential alleles have not been formulated. RHOADES (1941) has already proposed the concept of a continuous spectrum of mutation frequency of which only a few points may be recognized.

Recent genetic research has revealed the complexity of several genes. The components of the *R* (STADLER 1951) and the *A* (LAUGHNAN 1948) alleles in maize have been separated by their mutability and physiological action where recombination tests at first failed to subdivide the locus. Intensive studies of large numbers of progeny among allelic recurrences in *E. coli* (LEDERBERG 1951), Neurospora (BONNER 1951; GILES 1951), and Drosophila (GREEN and GREEN 1949; LEWIS 1951; TAKU-KOMAI 1950) have also revealed a fine structure in the loci involved.

In *E. coli* spontaneous or induced changes in mutability pattern at the Lac_1 complex invariably involved alterations to more stable types. The miniature alleles in *Drosophila virilis* which differ in their mutability, however, are able to mutate to either more stable or unstable forms (DEMEREC 1941). A similar situation exists for an unstable Mal^- mutant in *E. coli* (LEDERBERG *et al.* 1951).

The intrinsic factors determining the degree of mutability are often autonomous, i.e., appear to reside within the locus. Other genotypic influences have been discovered, however. The existence of at least two mutability modifiers of the *R* locus has also been recently suspected (STADLER 1949). The most clear-cut specific intergenic regulator of mutability has been described by RHOADES in Dotted maize (RHOADES 1938, 1941, 1945).

Technical difficulties have precluded a biochemical analysis of the mutability interactions in maize. On the other hand, bacteria would be eminently suitable for exploring such effects. Modifiers that suppressed the formation of Lac^+ reversions in unstable Lac^- strains were readily found. Closer examination revealed that the stable Lac^- types had remained potentially mutable, but the mutants could not be expressed in combination with certain alleles at other loci. In strains W-516, W-744, and W-750, a reduced selective advantage interfered with the expression of Lac^+ mutants. In addition, Gal^- modified the phenotype of the Lac^- cells by enhancing galactosidase activity. These genetic factors may be summarized as affecting the conditions under which the Lac^+ mutants are favored. No gene-induced alteration of the intrinsic mutation rate of another gene was discovered in this study.

As far as could be determined, the Lac^+ derivatives of each mutable Lac^- strain were reverse mutants. Suppressor loci were found, however, by selecting lactose fermenters from stable strains. These loci are latent in the wild type Lac^+. They can be detected as mutations which mimic wild type by suppressing the effect of Lac_1^- mutation.

The two suppressors reported in Neurospora are each less efficient than the

comparable reversions (HOULAHAN and MITCHELL 1947; GILES 1950). This was also true for several Lac_1^+ mimic factors. The Lac^+ of W-716 which most closely resembled Lac_1^+ was rapidly eliminated in a lactose-free medium. The inefficiency of suppressor mutants may perhaps be ascribed to either genic inefficiency or to imbalance with the rest of the genotype.

Duplication of a locus has been postulated as one mechanism for the origin of suppressors and pseudoalleles (SCHULTZ and BRIDGES 1932; HOULAHAN and MITCHELL 1947; STEPHENS 1948). One may visualize the transfer of such a duplication to another location, followed by gradual modification of its original activity and eventual evolutionary divergence both in microorganisms (LEDERBERG 1948b) and in higher forms (STEPHENS 1951).

SUMMARY

A series of recurrent Lac_1^- mutants was classifiable by the rates of reversion to Lac^+. Four major types were recognized on the basis of the average number of Lac^+ papillae per colony under standard conditions. All alterations of mutability, whether spontaneous or induced, were in the direction of greater stability. The papillae isolated from the mutable Lac^- strains were back-mutations as proven by backcrosses to wild type. However, stable strains gave rise to mimic reversions (suppressors) phenotypically less competent than wild type.

The primary differences in mutability were inseparable from the locus in recombination experiments. Other loci controlling apparent mutability were implicated in secondary stable types isolated from mutable strains. Further studies showed that the second mutation lowered the selective advantage of potential Lac^+ mutants but did not necessarily modify the intrinsic mutability directly.

The subdivision of the Lac_1^- locus into two components, *a* and *b*, was revealed by the rare occurrence of Lac^+ recombinants among intercross progeny. a^-b^+/a^-b^- heterozygotes were phenotypically lactose-negative. On the other hand, the a^+b^+ crossovers, both in haploids and in heterozygotes were phenotypically lactose-positive, indicating a "position effect."

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